FINAL TECHNICAL REPORT

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GRANT TITLE: Ecology and Evolution of Bioluminescent Bacteria

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OBJECTIVE: To determine and assess factors affecting population dynamics of bioluminescent bacteria in estuarine, coastal, and near-coastal seawater.

ACCOMPLISHMENTS:

1. Population Dynamics of Bioluminescent Vibrios. The numbers of bioluminescent bacteria (BLBs) in coastal waters fluctuate markedly throughout the year. Maximum densities are found in Summer months with a minimum in the Winter/Spring. Previous studies have indicated a positive correlation between water temperature and the densities of bioluminescent bacteria. Although vibrios are susceptible to chill damage, water temperature alone is insufficient to explain the kinetics of decrease in bioluminescent bacteria in the Fall and repopulation of coastal waters during the Spring. The decline of BLBs precedes the decrease in coastal water temperature in late Fall. Similarly, the increase in BLB numbers lags the increase in coastal water temperatures in the Spring by several months. Repopulation by BLBs occurs in late May, while water temperatures had been in the 18°C to 24°C range since early March.

2. Potential Sources of BLBs. Two phenomena previously were postulated to contribute to the repopulation of coastal waters with BLBs. First, BLBs could be concentrated up the food chain and become part of the intestinal microbial population of shorebirds. After wintering in warmer areas, and collecting BLBs, shorebirds might contribute to repopulating coastal waters during migration along the Atlantic Flyway. While vibrios ($> 10^7$ bacteria per gram) and BLBs (up to $2X10^6$ per gram) were recovered from shorebird and seagull excrement, the discrepancy between the timing of the migration and the repopulation of coastal waters with BLBs leads to the conclusion that these two phenomena are not linked causally. A second hypothesis was that BLBs are reintroduced into coastal waters through the inshore migration of invertebrates and fish. BLBs are present in the intestines of invertebrates and fish, as well as being parasites of crustaceans. According to this scenario, repopulation of coastal waters with BLBs occurs once a sufficient density of invertebrates and fish move inshore during early to mid-May. This hypothesis has not been directly tested. Collection of amphipods infected with BLBs is consistent with this proposal. While bioluminescent amphipods can be regularly collected during the summer months, and as late as November, the onset of their appearance is in late May, coincident with the onset of repopulation of coastal waters with BLBs. Releases of BLBs into seawater from the bioluminescent amphipods were observed, ranging from $2x10^4$ to $> 10^7$ BLB per ml. Three

different species of BLBs were recovered from the infected amphipods, including the normally encountered *V. harveyi*, and two human pathogens: *V. vulnificus* and *V. parahaemolyticus*, a common food-poisoning organism, not previously known to be luminescent.

3. <u>Expression of Bacterial Bioluminescence Under Anaerobic Conditions.</u> The presence of BLBs in the intestines of birds and fish leads to the question of whether bioluminescence can have any function in these oxygen-poor environments. Bacterial luciferase is unusual in that it can utilize oxygen at submicromolar concentrations and thus potentially may serve as an alternate electron acceptor when the bacteria are present in anaerobic environments. This possibility was tested by growing a number of different species of BLB and isolates from seagull excrement under anaerobic conditions using a non-fermentable carbon source (glycerol). Only low levels of luciferase expression were obtained. However, after addition of DMSO, expression increased 3 to 5 fold. DMSO is known to induce the fumarate-nitrate reductase (*fnr*) system in *E. coli* which permits utilization of alternate electron acceptors under anaerobic conditions. As in *E. coli*, DMSO was readily reduced to dimethyl sulfide by most vibrio isolates. Vibrios may play a role in the reduction of DMSO observed in marine sediments. Nitrate substituted for DMSO in increasing luciferase expression under anaerobic conditions.

<u>SIGNIFICANCE</u>:

1. The population dynamics of BLBs in coastal waters is complex. It is not causally linked to temperature changes or to the passage of migratory birds. The factor(s) responsible for repopulation of coastal waters in late Spring may have relevance to those concerned with Public Health issues.

2. The BLBs infecting amphipods are not one species, but are several species of closely related vibrios, including two human pathogens. The release of BLBs from dead amphipods may contribute to the repopulation of coastal waters.

3. The increase bioluminescence observed in the presence of inducers of the *fnr* system suggests that bioluminescence may in part be regulated by the *fnr* system.

4. The *fnr* operon may be involved in the reduction of DMSO by bacteria in marine sediments.

<u>PUBLICATIONS AND REPORTS</u>:

1. O'Kane, D. J., and B. G. Gibson (1993). "Cryopreservation of bioluminescent bacteria". *Proc. VIIth International Symposium on Bioluminescence and Chemiluminescence*. John Wiley, Chichester, U.K., in press.

2. O'Kane, D.J., and B. G. Gibson (1993). "Thermal stability of bacterial bioluminescence". *Proc. VIIth International Symposium on Bioluminescence and Chemiluminescence*. John Wiley, Chichester, U.K., in press.

3. O'Kane, D. J., "Characterization of bioluminescent bacteria from infected amphipods", in preparation for submission to *J. Biolum. Chemilum.*y

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